Cat. # R010A

For Research Use

TaKaRa

PrimeSTAR® HS DNA Polymerase

Product Manual

v201307Da

Table of Contents

I. Description

PrimeSTAR HS DNA Polymerase, developed by Takara Bio, is a unique high fidelity DNA polymerase that allows high efficiency PCR amplification of DNA. Excellent performance is achieved by superior proofreading ability due to robust 3' → 5' exonuclease activity. Furthermore an antibody-mediated hot start (HS) formulation prevents false initiation events during the reaction assembly due to mispriming and primer digestion. When used with the optimized reaction buffer, PrimeSTAR HS DNA Polymerase provides high fidelity, high sensitivity, and high specificity amplification for applications such as DNA amplification from cDNA libraries.

II. Components (for 200 reactions)

PrimeSTAR HS DNA Polymerase (2.5 units/μl) ..... 100 μl
5X PrimeSTAR Buffer (Mg^{2+} plus) ＊ ..... 2 x 1 ml
dNTP Mixture (2.5 mM each) ..... 800 μl

＊ : Mg^{2+} concentration: 5 mM (5X)

III. Storage

-20℃

IV. Features

A. Accuracy

The frequency of mutations introduced using PrimeSTAR HS DNA Polymerase was analyzed by DNA sequence analysis.

[Method] Eight arbitrarily selected GC-rich regions were amplified with PrimeSTAR HS DNA Polymerase and other enzymes, using *Thermus thermophilus* HB8 genomic DNA as a template. Each PCR product (approx. 500 bp each) was cloned into a suitable plasmid. Multiple clones were picked per region for sequence analysis.

[Result] Sequence analysis of DNA fragments amplified using PrimeSTAR HS polymerase had only 12 erroneous nucleotides in 249,941 total nucleotides. This fidelity is higher than that obtained with a high fidelity enzyme from Company A, and it is 10 times higher than *Taq* DNA polymerase.

The above method is the most realistic method to investigate the mutation frequency. Based on the sequence analysis results, it is recommended that PrimeSTAR HS DNA Polymerase be used for PCR amplification that requires the extreme accuracy.

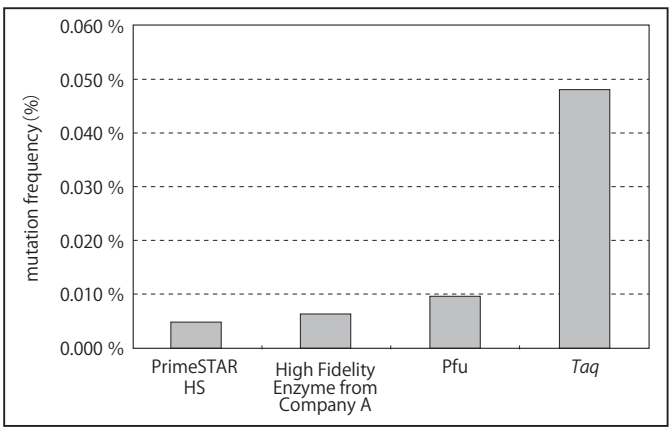
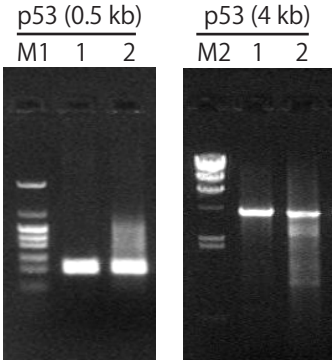


Figure 1. Fidelity comparison with other enzymes

B. High Priming Efficiency- Short Annealing Time

PrimeSTAR HS DNA Polymerase possesses extremely high priming efficiency. Thus a short annealing time, only 5 or 15 seconds, can be used to achieve highly specific amplification. Refer to "VI. PCR Conditions" for reaction condition guidelines.



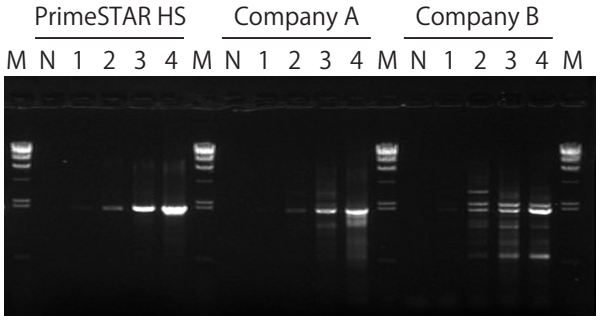
1 : Annealing at 55℃ for 5 sec.
2 : Annealing at 55℃ for 30 sec.
M1 : pHY Marker
M2 : λ-*Hin*d III digest

Template : Human genomic DNA 100 ng
50 μl PCR reaction
3 step PCR Method, 30 cycles

C. High Amplification Efficiency

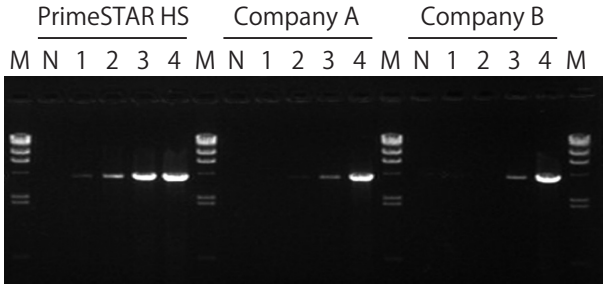
1) Amplification efficiency was compared against Company A's and Company B's high fidelity enzymes. (Target: Human DCLRE1A gene [2 kb], *E. coli* [4 kb])

Reaction mixtures were prepared and PCR cycling conditions were performed according to each supplier's protocol (50 μl PCR reaction).



< Human DCLRE1A gene [2 kb] >

N : No Template
1 : human genomic DNA 100 pg
2 : human genomic DNA 1 ng
3 : human genomic DNA 10 ng
4 : human genomic DNA 100 ng
M : λ-*Hin*d III digest



< *E. coli* [4 kb] >

N : No Template
1 : JM109 genomic DNA 1 pg
2 : JM109 genomic DNA 10 pg
3 : JM109 genomic DNA 100 pg
4 : JM109 genomic DNA 1 ng
M : λ-*Hin*d III digest

The above results demonstrate that PrimeSTAR HS DNA Polymerase provides excellent amplification efficiency with higher specificity than other suppliers' high fidelity enzymes.
In addition, detection sensitivity was higher by one order of magnitude.

2) Amplification of various DNA fragment sizes, using human genomic and *E. coli* genomic DNA as the template.

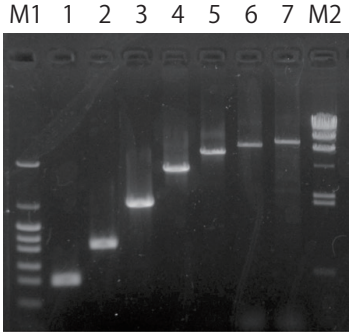
Template: Human genomic DNA [50 ng/50 μl PCR reaction]
Thermal cycler: TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)
PCR conditions:

0.5 - 6 kb: 3 step PCR Method

98℃	10 sec.	30 cycles
60℃	5 sec.	
72℃	1 min. /kb	

7.5 - 8.5 kb: 2 step PCR Method

98℃	10 sec.	30 cycles
68℃	8 min.	

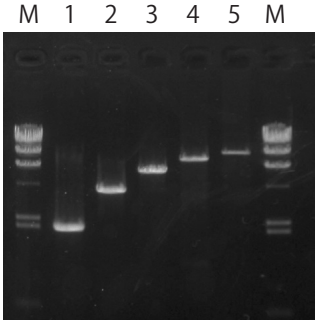


1 : 0.5 kb
2 : 1 kb
3 : 2 kb
4 : 4 kb
5 : 6 kb
6 : 7.5 kb
7 : 8.5 kb
M1 : pHY Marker
M2 : λ-*Hin*d III digest

Template: *E. coli* genomic DNA [100 pg/50 μl PCR reaction]
Thermal cycler: TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)
PCR conditions:

3 step PCR Method

98℃	10 sec.	30 cycles
60℃	5 sec.	
72℃	1 min. /kb	



1 : 2 kb
2 : 4 kb
3 : 6 kb
4 : 8 kb
5 : 10 kb
M : λ-*Hin*d III digest

D. High Thermostability

1) Residual activity was approximately 80% after 30 cycles using a 3 step PCR protocol (98℃ 10 sec.; 55℃ 15 sec.; 72℃ 4 min.)
2) Residual activity was approximately 85% after 30 cycles using a 2 step PCR protocol (98℃ 10 sec.; 68℃ ; 4 min.)

V. General Composition of PCR Reaction Mixture (total 50 μl)

	Volume	Final conc.
5X PrimeSTAR Buffer (Mg^{2+} Plus)	10 μl	1X
dNTP Mixture (2.5 mM each)	4 μl	200 μM each
Primer 1	10 - 15 pmol	0.2 - 0.3 μM
Primer 2	10 - 15 pmol	0.2 - 0.3 μM
Template	<200 ng	
PrimeSTAR HS DNA Polymerase (2.5 units/μl)	0.5 μl	1.25 units/50 μl
Sterilized Distilled Water	up to 50 μl	

＊ The PCR reaction mixture can be prepared at room temperature. However, keep each of the reaction components on ice while preparing the reaction mixture.

VI. PCR Conditions

(A) 3 step PCR

98℃ 10 sec.
55℃ 5 sec. or 15 sec.
72℃ 1 min. /kb
] 30 cycles

(B) 2 step PCR

98℃ 10 sec.
68℃ 1 min. /kb
] 30 cycles

First try 3 step PCR when using PrimeSTAR HS DNA Polymerase.

- Denaturation conditions: 98℃ 5 - 10 sec.
  Alternatively, if a denaturation temperature lower than 94℃ is used, set the denaturation time to10 - 15 sec.
- Annealing temperature: Initially, try 55℃ (optimization may be required.)
- Annealing time: Annealing time is dependent upon primer Tm values. Calculate primer Tm values using the following formula (＊)；
  When Tm ≧ 55℃ Set for 5 sec.
  When Tm ＜ 55℃ Set for 15 sec.

(＊) Tm value (℃) ＝ 2 (NA ＋ NT) ＋ 4 (NC ＋ NG) － 5

The above Tm value formula is valid for primers whose lengths are ≦ 25 mer. For primers longer than 25 mer, an annealing time of 5 sec. should be used.

[Important Note]

Because the priming efficiency of PrimeSTAR HS DNA Polymerase is extremely high, an annealing time of 5 sec. or 15 sec. should be used. Longer annealing times may cause smearing of PCR products.

Try a 2 step PCR protocol if smeared DNA products are observed by agarose gel electrophoresis of amplified DNA obtained using 3 step PCR or when using primers with Tm values ≧ 70℃. Please refer to "VII. Optimization of parameters" and "IX. Troubleshooting" for additional PCR condition recommendations.

VII. Optimization of Parameters

To obtain the best PCR results, it is important to optimize reaction parameters when using PrimeSTAR HS DNA Polymerase.

1) Enzyme amount

In general, 1.25 units of enzyme per 50 μl reaction is recommended. Depending upon the amplified fragment size, purity, and amount of template, the amount of enzyme may be adjusted accordingly.

2) Template DNA amount

Recommended template DNA amounts (assuming a 50 μl reaction):

Human genomic DNA :	5 ng - 200 ng
E. coli genomic DNA :	100 pg - 100 ng
cDNA library :	1 ng - 200 ng
λ DNA :	10 pg - 10 ng
Plasmid DNA :	10 pg - 1 ng

Avoid excess template DNA, which can lower enzyme reactivity.

Templates containing Uracil, such as bisulfite-treated DNA, cannot be amplified with this enzyme.

3) dNTP and Mg^{2+} concentration:

Because dNTPs have a chelating effect, excess dNTPs will lower the effective Mg^{2+} concentration in the reaction mixture. The supplied 5X PrimeSTAR Buffer provides 1 mM final Mg^{2+} concentration that has been optimized for use with a 200 μM final dNTPs concentration in the reaction mix. Thus the concentration of dNTPs should not be modified.

Note: Do not substitute dUTP for dTTP in the PrimeSTAR HS reaction mix. The use of dUTP drastically decreases enzyme reactivity.

4) Primer and PCR condition

The use of commercially available primer design software, such as OLIGO™ Primer Analysis Software (Molecular Biology Insights) is recommended for obtaining appropriate primer sequences that follow general primer design guidelines and can be tailored specifically for your template DNA.

Guidelines for Primer Design:

a) Primer length:	For general amplification of DNA fragments, 20 - 25 mer primers are suitable. Exact PCR conditions should be determined by referring to "VI. PCR conditions".
b) Modified bases:	Never use primers containing inosine (I) with PrimeSTAR HS DNA Polymerase.
c) Degenerate primers:	Degenerate primers may be used with PrimeSTAR HS DNA Polymerase.

5) Annealing conditions

Annealing conditions should be determined by referring to "VI. PCR Conditions". When low yield of amplified PCR product is obtained, troubleshoot as follows:

<Smearing and/or extra bands appear on agarose gels>

i) Shorten the annealing time. For example, decrease time from 15 to 5 sec.
ii) If the annealing time is already 5 sec., then raise the annealing temperature to 58 - 65℃
iii) Try 2 step PCR.

<Target product is not amplified.>

i) Extend the annealing time. For example, increase time from 5 sec. to 15 sec.
ii) Lower the annealing temperature to 50 - 53℃ .

VIII. Electrophoresis, Cloning, and Sequencing of Amplified Products

1) Electrophoresis of the amplified product

TAE Buffer is recommended for agarose gel electrophoresis of amplified products that are obtained using PrimeSTAR HS DNA Polymerase.

Note: Use of TBE Buffer may result in DNA band patterns which are enlarged at the gel bottom.

2) Termini of amplified products

Most PCR products amplified with PrimeSTAR HS DNA Polymerase have blunt end termini. Accordingly they can directly be cloned into blunt-end vectors (if necessary, phosphorylate before cloning), but can not be cloned directly into T-vectors. Mighty Cloning Reagent Set (Blunt End) (Cat. # 6027) is recommended for cloning into blunt-end vectors.

3) Restriction enzyme reaction

Prior to performing restriction enzyme digestion of amplified PCR products, remove all traces of PrimeSTAR HS polymerase from the reaction mix by phenol/chloroform extraction or DNA extraction by NucleoSpin® Gel and PCR Clean-up (Cat. #740609.10/.50/.250). In particular, the restriction site produced with enzymes arising 3′ -protruding cleavage sites, such as *Pst* I, may be digested by residual 3′ → 5′ exonuclease activity of PrimeSTAR HS DNA Polymerase, resulting in deletions.

4) Direct sequencing

Perform phenol/chloroform extraction or DNA extraction using NucleoSpin® Gel and PCR Clean-up (Cat. #740609.10/.50/.250) of PCR products prior to direct sequencing and ensure inactivation of 3' → 5' exonuclease activity.

IX. Troubleshooting

1) Problem: No amplified product, or low amplification efficiency.

Solution:
i) Extension time: Use an extension time >1 min./kb.
ii) Annealing time: 15 sec.
iii) Annealing temperature: Lower temperature in decrements of 2℃. Alternatively, use a 3 step PCR protocol.
iv) Purity and quality of template DNA: Use an appropriate amount of template DNA; refer to "VII. Optimization of Parameters". Use a more highly purified template DNA.
v) Primer concentration: Test the final primer concentration in the range of 0.2 - 0.5 μM.

2) Problem: Extra bands appear or DNA smearing is observed during agarose gel electrophoresis.

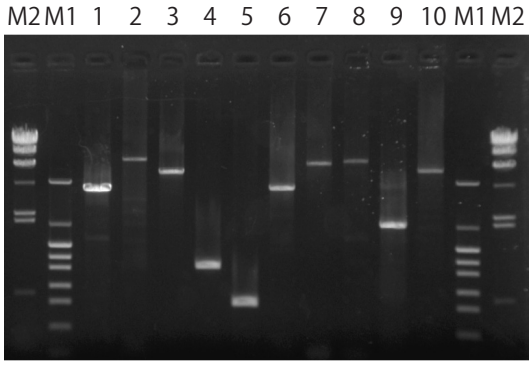
Solution:
i) Annealing time: 5 sec.
ii) Annealing temperature: Raise the temperature in increments of 2℃. Alternatively, perform cycling using the 2 step PCR Method.
iii) Extension time: 1 min/kb. Avoid excessive extension times.
iv) Template DNA: Use an appropriate amount of template DNA; Avoid excessive amounts of template DNA.
v) Cycle number: 25 - 30 cycles.
vi) Enzyme amount: Decrease amount of enzyme within the recommended range; minimum recommended amount of PrimeSTAR HS polymerase is 0.625 units/50 μl reaction mix.
vii) Primer concentration: Determine the optimal concentration in the range of 0.2 - 0.3 μM (final conc.).

X. Experimental Example

Amplification of various lengths from human genomic DNA using a single PCR reaction condition.

The results demonstrate that PrimeSTAR HS DNA Polymerase can efficiently amplify various targets ranging from 0.5 - 8.5 kb in length using the same PCR conditions.

Template: Human genomic DNA 100 ng/50 μl PCR
PCR condition: 98℃ 10 sec. / 68℃ 8 min.] 30 cycles



1 : DCLRE1A 4 kb
2 : β-globin 8.5 kb
3 : β-globin 6 kb
4 : DCLRE1A 1 kb
5 : p53 0.5 kb
6 : p53 4 kb
7 : β-globin 7.5 kb
8 : DCLRE1A 8 kb
9 : DCLRE1A 2 kb
10 : p53 6 kb

M1 : pHY Marker
M2 : λ-*Hin*dIII digest

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[P1] PCR Notice
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[L15] Hot Start PCR
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[M54] PrimeSTAR HS DNA Polymerase
This product is covered by the claims of U.S. Patent Nos. 7,704,713 and its foreign counterparts.

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